# Microarray-Based Resequencing of Multiple *B. anthracis* Isolates

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# Report Documentation Page

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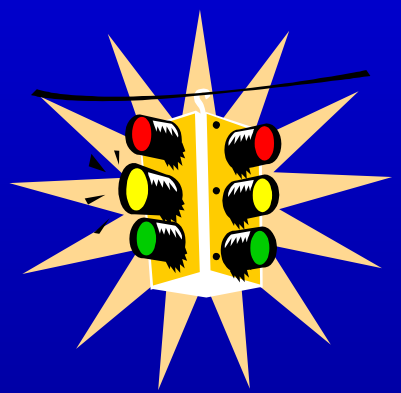
Prescribed by ANSI Std Z39-18

# A Layered Approach: Levels of BW Testing



**PRESUMPTIVE**
(Hand-held assays)

Detect to Treat

2 tests

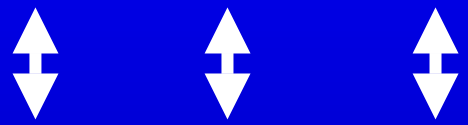
**CONFIRMATORY**
(ELISA's, PCR, Culture)

<24 hours

**DEFINITIVE**
(Technical Reachback, Monthly QC)
(Full-scale analytical work up by the experts)

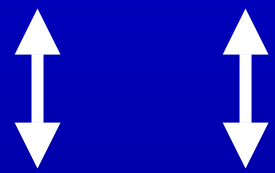
# Navy BW Testing Assets

## Forward Deployed Forces/ Small Ships

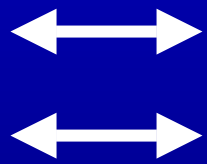


**NEPMU's** *(FD-PMU's)*

**CV/CVN's** *(LHA/LHD's & T-AH's)*



**USAMRIID**
**CDC**
**AFIP**



**BDRD (NMRC)**

- Coronado
- Enterprise
- George Washington
- Harry S. Truman
- John F. Kennedy
- Theodore Roosevelt
- Abraham Lincoln
- Carl Vinson
- John C. Stennis
- Kitty Hawk
- Nimitz
- Bataan
- Iwo Jima
- Kearsarage
- Nassau
- Saipan
- Wasp
- Bonhomme Richard
- Boxer
- Essex
- Peleliu
- Tarawa
- Comfort
- Mercy

# How Can We Detect and Identify BW Agents?

Genotype markers known to show variation

- Fixed species specific variants, previously identified
- Rapid detection of a small number of sites
- Example: Real-Time PCR (Confirmatory Lab)

DNA sequence regions/genomes of interest

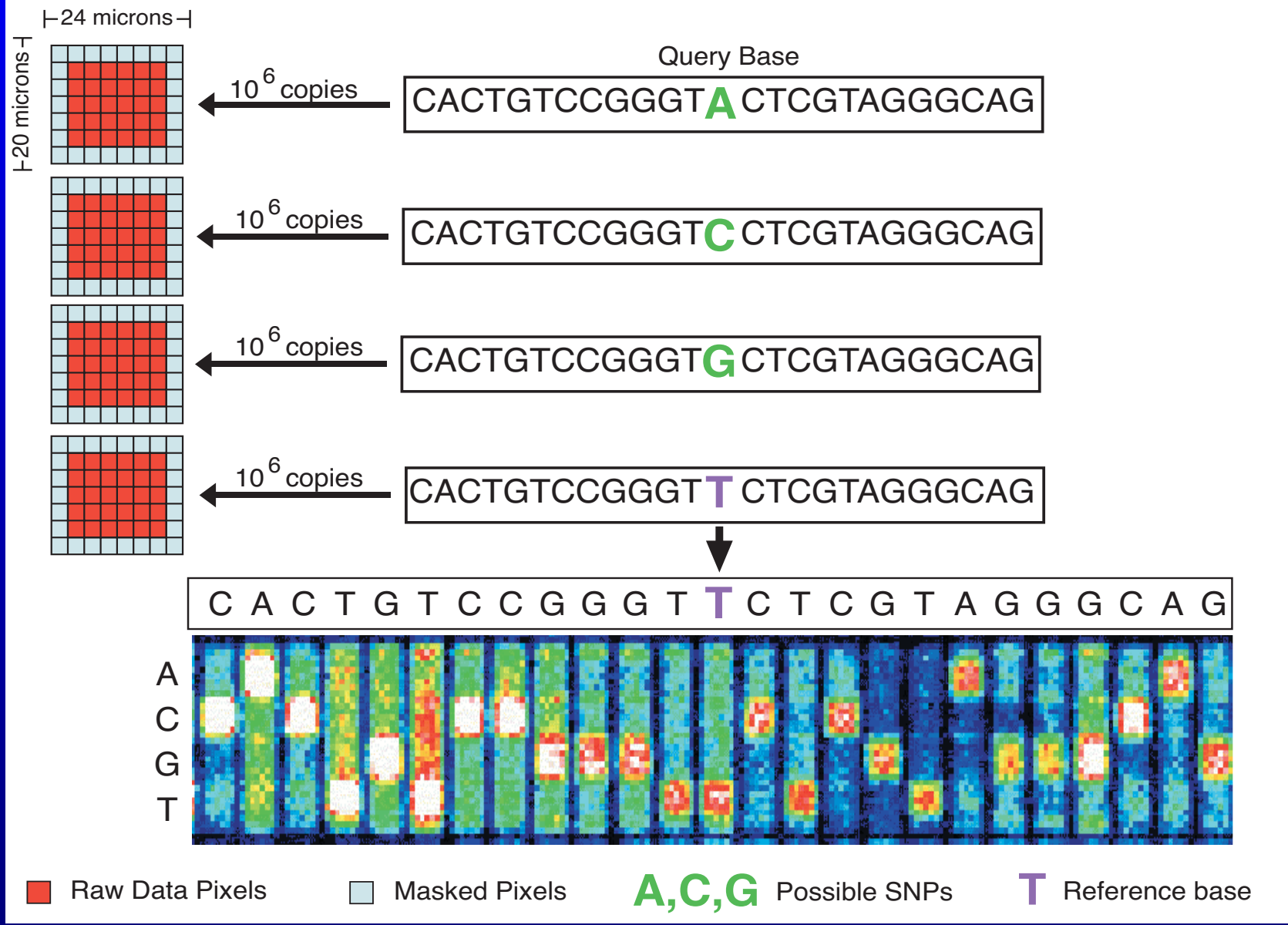
- Maximally informative:

The sequence is the genotype!

- Detects common and rare variants
- Strain identification/origin (Definitive Lab)

The future detection and identification of BW agents will increasingly depend upon DNA sequencing technologies

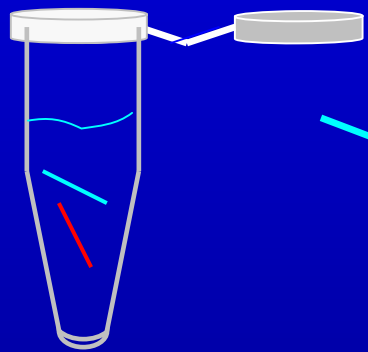
# Design of Resequencing Arrays



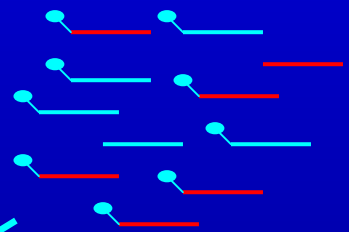
# Resequencing Assay

Long PCR/Whole Genome Amplification

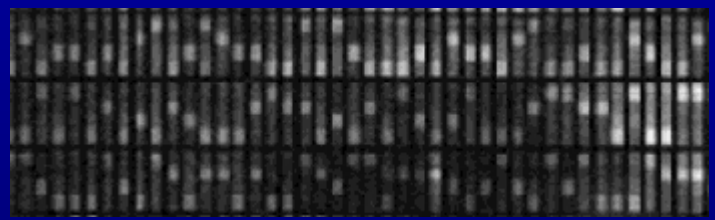
PCR products pooled by individual; DNAse I treated (50 bp average)



DNA fragments biotinylated



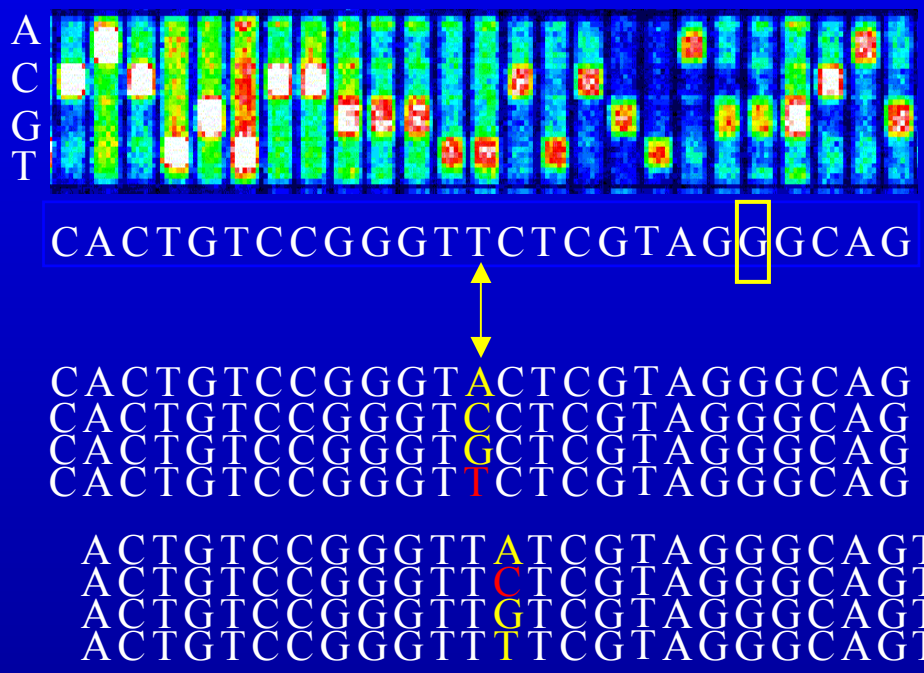
Tagged fragments hybridized to an oligonucleotide array; stained with streptavidin phycoerythrin



Analyzed by ABACUS to detect variation

# Resequencing *B. anthracis*

- 29.5 kb of unique sequence per chip.
- Each array has ~320,000 features.
- Forward and reverse strands tiled.
- 1 design, 6 LPCR assays
- pXO1, pXO2, Main Chromosome: All or part of 32 genes
- lef, pag, cap, vrr, rpoB, sasB




❖ How certain are we of this G ?

# ABACUS: An Automated Statistical Algorithm for Base/Genotype Calling

- Within any given feature, florescence intensities of individual pixels are assumed to be independent and identically distributed Gaussian variables.
- Forward and reverse strands are treated as independent replicates (with different parameters).
- All parameters are fit by maximum likelihood.
- 5 models for haploid data (null,A,C,G,T).
- 11 models for diploid data (null, AA,CC,GG,TT,AC, AG, AT, CG, CT, GT).
- Neighborhood quality rules are used.

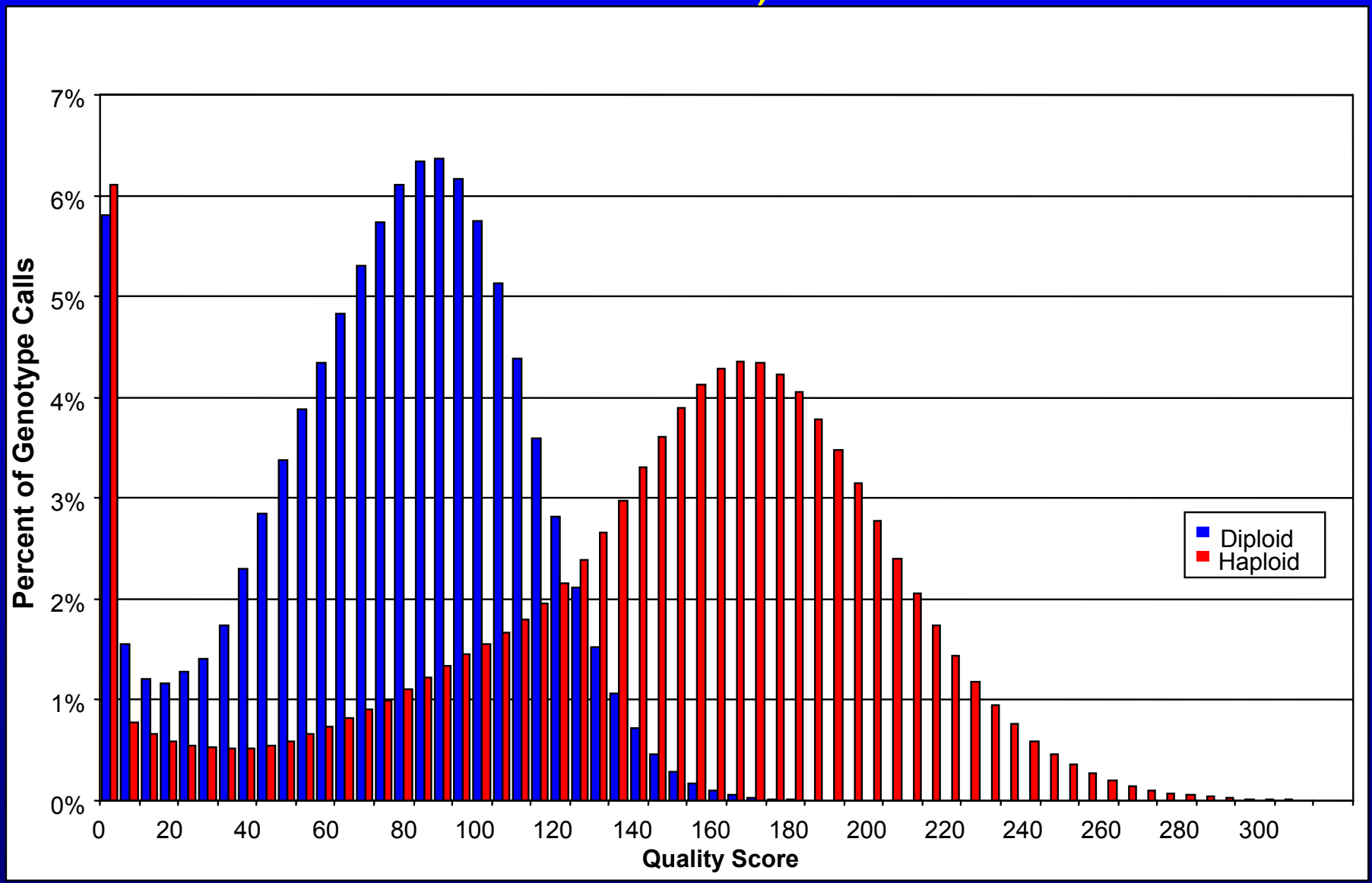
# ABACUS Assigns Quality Scores to Each Base/Genotype Call

- A *Quality Score*, the difference between the $\log_{10}$ likelihood of the best fitting and second best fitting model, is assigned to each genotype.
- Information from both the forward and reverse strands is incorporated into the *Quality Score*.
- Genotypes inferred only when a *Quality Score* threshold is reached.

For more detail, see Cutler, DJ, Zwick, ME *et al.*

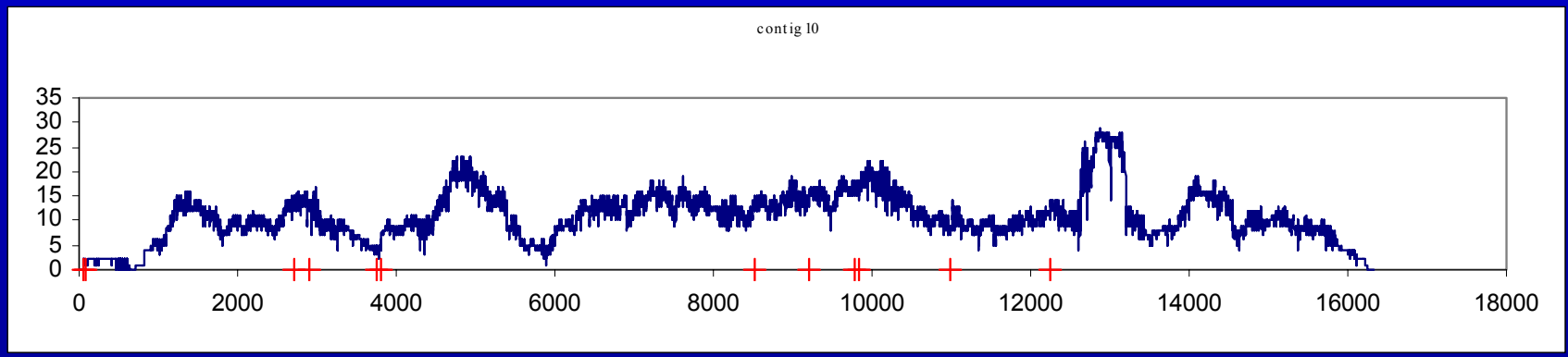
Genome Res. 2001 11: 1913-1925

# Distribution of *Quality Scores* (Human Data)



# Haploid ABACUS Base Calls Are Highly Accurate (QS>30)

- LPCR fragments hydrosheared
- Individual 8 from FMR1
- Subcloned with end-repair into PUC Library
- Single Pass sequenced with M13 primers
- At least 6x




- 17,423 bp with at least 6x coverage, all identical to ABACUS calls
- At 2x coverage, an additional 4,081 bp, with 1 difference from ABACUS calls

# ABACUS Genotype Calls Are Highly Repeatable

- Haploid
  - 0 differences / 841,236 sites (QS>30)
- Diploid
  - 0 differences / 812,944 homozygotes (QS>30)
  - 0 differences / 351 heterozygotes (QS > 30)
- Implies a phred score of at least 54

# *B. anthracis* Resequencing Experiment

- Chips Hybridized and Scanned: 114
  Successful: 112
  Experimental Failure: 2

- *B. anthracis* Isolates Analyzed: 59
  Replicated: 53 (106 chips)
  Single Analysis: 6 (6 chips)

# Microarrays Can Generates Vast Amounts of Sequence Data

- Raw Sequence Generated

Bases Called: 3,052,254

Total Possible Bases: 3,271,744

Call Rate: 93.3%

- Variant Sites Discovered

38 Single Nucleotide Polymorphisms (SNPs)

16 of 38 SNPs singletons

22 SNPs found more than once

# Anthrax Resequencing is Highly Replicable

| | |
|---|---|
| Total Comparisons | 1,420,583 |
| Total Bases Called | 2,897,098 |
| Total Discrepancies | 1 |

- Suggests error rates of less than 1 per million
- Quality Score Threshold: 31
- Sequences on chip:  34.7% GC Content

# How different are two *B. anthracis* isolates?

- Variation Estimates
- Tajima's Estimate of Theta: $1.6 \times 10^{-4}$
- Watterson's Estimate of Theta: $2.9 \times 10^{-4}$

- Two Isolates of *B. anthracis* are expected to differ at between:

~924 (Tajima)

and

~ 1606 (Watterson)

Resequencing can uniquely identify *B. anthracis* isolates

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# Points of Contact

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# Assessing ABACUS Performance

- Replicability: Comparison of haploid/diploid replicates by independent:
  - PCR amplification of genomic DNA
  - Manufacture of resequencing arrays (distinct wafers)
  - Hybridization of amplified DNA to chips
  - ABACUS genotype calls
- Accuracy: Independent Genotyping/DNA Sequencing

All genotyping technologies should be assessed using these criteria

# Diploid ABACUS Genotype Calls Are Highly Accurate (QS>30)

- Homozygous genotypes
  - 0 differences / 1,515 genotypes (100% correct)
- Heterozygous genotypes
  - 3 differences / 423 genotypes (99.3% correct)

- Two of the three differences were in a single LPCR fragment
- All three differences were at high frequency sites
- Chips called heterozygote, sequencing called homozygote

Probable Cause: Sample Mixing